Home Office

NON-TECHNICAL SUMMARY

Immune responses to new vaccine platforms

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

mRNA treatments, vaccine responses, infection, cancer, toxicity

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To evaluate vaccine responses and toxicities of new platform-generated vaccines

A retrospective assessment of these aims will be due by 28 February 2030

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There have been big improvements in how well mRNA treatments work lately. mRNAs are special messengers that deliver instructions for our cells to make specific proteins, we can design them to contain these instructions. The proteins produced will help us fight off certain diseases, such as viruses, e.g., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We can use these designed mRNA for vaccination and/or therapy. We have witnessed the success of mRNA treatments through mRNA SARS-CoV-2 vaccines, which have helped us overcome COVID-19 pandemic. In addition to this, recent work has shown that mRNA treatments are very promising in the development of pancreatic cancer medicines, showing that administration of personalised mRNA therapy targeting tumour proteins, in combination with other drugs, can reduce and delay tumour recurrence in patients. With the recent discoveries showing the promise of mRNA treatments, we hope to develop new mRNA vaccines to protect against infectious diseases as well as cancer. We will need to assess the capability of this and other vaccine platforms (e.g., protein-based vaccines) in mice, the immune response they generate and any potentially harmful side effects they may have before moving to their use in medical treatments.

What outputs do you think you will see at the end of this project?

The outputs will be data demonstrating the effectiveness of novel vaccines for infectious diseases and cancer, the identification of harmful side effects if any and the discovery of the best indicators of vaccine success that can be subsequently used in clinical trials to measure responses. It will also generate new information on the underlying mechanisms of vaccine responses. These data will lead to scientific publications, will be disseminated through national and international meetings and may generate Intellectual Property.

Who or what will benefit from these outputs, and how?

In the short term, we anticipate scientific publications to arise from these studies, putting together information to show why we should use the findings in real medical treatments. There will also be benefits from identifying those treatments that are not effective or have undesired effects, avoiding future patients from being exposed to non-beneficial, harmful, treatment.

In the longer term, we hope to increase our understanding of vaccination and give reasons why they should be used, in infectious diseases and cancer.

How will you look to maximise the outputs of this work?

The proposed studies require collaboration with pharmaceutical/biotech companies who are developing some of the vaccines we wish to test, and we have long-standing and productive collaborations that will continue to give us access to the best treatments that are commercially viable (and therefore developable).

We will disseminate new knowledge via publication. We would like to publish unsuccessful approaches if there were an appropriate route (e.g., F1000Research), as long as the data were not restricted as part of a commercial agreement.

Species and numbers of animals expected to be used

- Mice: 19040

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the species with the lowest sensitivity of the nervous system likely to produce data predictive of the effect in humans. Also, the majority of animal models to evaluate the immune system and vaccine responses have been developed in mice. This means many resources are already available for mouse models.

Studies of the effects of vaccines on the body and/or on the whole tumour in its complex environment have to be performed in live animals. Vaccination studies usually require adult mice to match the biology of the human disease, in some cases aged mice will be used to mimic vaccination in old people. We will use genetically modified mice to either alter a natural gene of interest or to express a “marker” gene that enables the detection of certain cell types.

Typically, what will be done to an animal used in your project?

Approximately 37% of the mice will be used in breeding protocols to produce mice with the correct genes for use in experiments and 63% of the total mice will be used in experimental procedures.

Most of the experimental mice (~77.3% of the total for experimental procedures) will either be vaccinated or be implanted with a tumour under the skin on the flank (or occasionally 2 tumours, one on each side).

Vaccines will be generally administered using the same routes as in humans. They will be directly injected into the muscle (intramuscular) or the skin (intradermally). Other routes of delivering may be used, such as directly into a vein using a needle (intravenously), under the skin (subcutaneously) or into the peritoneal cavity (the one around the tummy, intraperitoneally). In humans, vaccines are often administered intramuscularly or intradermally and we will primarily explore the use of these routes to refine and increase the clinical relevance of our vaccination protocols.

Vaccinated mice will be monitored for several weeks. Booster doses may be administered 3 to 6 weeks apart. The mice will be killed at the end of the experiments, with blood and tissues taken for subsequent analysis.

In the case of tumour-implanted mice experiments, they will be treated for up to several weeks with one or more anticancer drugs, anti-cancer vaccines, and a small number of mice may have the tumour under the skin removed surgically. Drugs may be administered either by mouth or by injection. The typical duration of study for an individual mouse, from the time of tumour implantation, would be 3 to 6 weeks. The mice will be killed at the end of the experiments, with blood and tissues taken for subsequent analysis to provide data on the effects of vaccines or drugs.

Towards the end of the project, some mice may be infected with viruses or bacteria (~4.3% of the total for experimental procedures) or be generated with naturally occurring tumours (~3.4% of the total for experimental procedures) to challenge vaccine efficacies.

A small portion of vaccinated mice will be infected with specific viruses or bacteria to test the efficacy of the new vaccine platforms for infectious diseases. Daily weight measurements will be performed before and after infection to monitor scientific and welfare endpoints. Some animals will be administered vaccines or certain substances, such as antibodies, to test the role of specific cell types within the host.

The microbiota, the bugs in our gut, may influence the response to vaccination. Mice may be exposed to microbiota stabilisers (e.g., antibiotics) and/or colonisation (faecal transplant or administration of specific bacteria strains by oral route). Blood sampling and non-invasive imaging under anaesthesia at any stage of the protocols may be required. A small number of mice may undergo intermittent or continuous administration of modified diets (e.g. high-fat/high-sucrose or similar), or fasting, to evaluate how the body responds to changes in energy and or nutrition. Mice may need to be bone marrow transplanted (~10.7% of the total for experimental procedures) or aged (~4.3% of the total for experimental procedures) to test vaccine platforms in relevant conditions such as ageing.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of experimental mice will experience transient discomfort from agents dosing/administration. New vaccine platforms are not expected to induce more than mild discomfort.

Subcutaneous tumours will rarely cause clinical signs but dissemination throughout the body is possible (most likely to the liver or lungs), inducing clinical signs such as breathing problems, hunched posture, decreased activity, possible immobility, signs of distress and weight loss. Some tumours may ulcerate. After the tumours become palpable they will be checked frequently for signs of ulceration. Should ulceration appear, it will be closely monitored. An animal will be killed if ulceration appears and is not healing, impedes a vital function (locomotion, vision, excretion, etc), or causes other clinical signs. If this should occur the animal will be humanely killed immediately. The weight of the mice will be monitored throughout the study. It is not expected for this to change significantly, however, possible weight gain as a result of developing tumours may happen. Body condition scoring will be included. They may also experience some adverse side effects from their anticancer drugs (usually seen as weight loss, diarrhoea, and/or reduction in their normal activity levels). Mice fed a high-fat diet may develop greasy coats which can lead to scratching and skin lesions. Ameliorating measures for itchy skin or lesions, such as nail trimming and/or green clay, will be undertaken. Other potential adverse effects of diet-induced obesity include impaired mobility and development of pre-diabetes.

A few animals will undergo short periods of general anaesthesia (e.g., for imaging). They may experience illness due to the presence of cancer (particularly those with pancreatic tumours), which may include weight loss, decreased activity, distress and build-up of fluid in the abdomen.

During infection, control animals without vaccination may experience clinical signs of infection, such as weight loss, reduced activity and motility, difficulty to breath, and in accessing water and food. Vaccinated mice will receive multiple treatments which carry a risk of adverse effects.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

73% of the mice are expected to experience mild severity.

25% are expected to experience moderate severity.

No more than 2% severe severity.

What will happen to animals used in this project?

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 28 February 2030

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The Regulatory Authorities (The Medicines and Healthcare Products Regulatory Agency (MHRA, UK), European Medicines Agency (EMEA, EU) and the Food and Drug Administration (FDA, USA)) will not allow agents to be tested in humans until they have been shown to be safe and effective in animals. Our research program involves exploiting the immune system to aid in vaccination strategies, and this requires the whole organism.

Currently, there are no non-animal alternatives that can be used to accurately predict or mimic the generation of an immune response to vaccination. This is why we need to conduct experiments on adult living mice, to explore the fundamental mechanisms of vaccine responses and identify potential ways to improve them.

Which non-animal alternatives did you consider for use in this project?

Whilst computer modelling and non-animal models of immune responses are ongoing and improving, these do not yet begin to replicate the complicated conditions and variables that exist in the immune system (e.g., complete structural, hormonal and cellular interactions).

We have generated a cellular system to test vaccine constructs before starting preclinical experimentation. In this system, we have produced cells to express specific molecules to evaluate immune cell responses. We have tested this system and we can predict which constructs are more likely to induce an immune response when we vaccinate adult living mice. This system, with appropriate validation within this programme, will help to replace animals as a first step for assessing vaccine constructs.

We do perform many experiments in cell lines grown in culture dishes, to screen different mRNA therapies, drugs and combinations to identify those most likely to be effective in mice/humans, and to investigate the mechanisms. We also use more complex mixed cultures– mixing cancer cells with other cell types that in the body may support the growth and drug resistance of tumours.

Why were they not suitable?

We do use complex cell cultures, however, these do not replicate the complicated conditions and variables that exist in the immune system. Once we have identified the best therapy options, we have to test them in the context of the whole animal. For example, it is very easy to kill cancer cells in a dish, but we have to identify those therapies that will not also be toxic to the normal body.

A retrospective assessment of replacement will be due by 28 February 2030

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We base our calculations on our previous data and experience. During the last year of our current licence we have performed experiments similar to the ones we plan for our next licence, this has helped us calculate more accurately the number of animals we will use. We also referred to published data of similar approaches and suggestions from our collaborators and colleagues. Additionally, we have used available online tools such as the Experimental Design Assistant (EDA, https://eda.nc3rs.org.uk) from NC3Rs to critique and analyse our experimental design.

We have used these estimates of a typical number of mice per study, multiplied by the maximum number of each type of study that we expect to perform over the 5 years, to estimate the total number of animals to be used in this programme. We have then calculated the expected number of mice on breeding protocols likely to be used to provide the required numbers of experimental mice. We will use approximately 60% of the mice from our breeding protocols and 40% wild type from external sources.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will use statistical tools to reduce the number of animals to use. We plan to control the variability through block randomisation, where some variables (e.g., genotype) are balanced at randomisation. This will help to control the distribution of confounding and predictive characteristics between compared groups. We will also use inbred strains to control inter-individual variation and standardisation of operative procedures and animal facility environmental conditions (e.g. temperature, diet, cage). When possible, we will perform experiments comparing multiple groups with a common control group.

We plan to use both sexes in our experimental groups, this will help improve the information we can retrieve from them and avoid unnecessary replication of experiments. When possible, a within comparison will be performed, we will sample before any treatment to determine baseline levels and compare to the treatment effect within the same animals.

Microsampling (taking tiny volumes) of blood permits multiple samples to be taken from each mouse for antibody levels assessments, so that measurements can be made at multiple times, reducing the number of mice required.

For complex transgenic mouse lines with multiple genetic alterations (alleles), as many alleles as possible will be carried homozygously - with both gene copies the same, so that each offspring

definitely inherits the genetic alteration. This reduces the breeding required to generate sufficient offspring carrying all the required alleles for completion of the goals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies will be performed to inform subsequent studies. Besides the use of the Experimental Design Assistant (EDA) from NC3Rs, a Bioinformatics/statistics core facility will provide advice on the study design, power calculations and statistical analysis.

If new breeding colonies are established, we will calculate the number of mice required to produce the number of mice with the correct genotype for the studies and check this against the estimate for that protocol. At the annual review, the total numbers used on each protocol will be reviewed in relation to the number permitted on the licence.

We will use the NC3Rs breeding and colony management guidelines to support our breeding strategies: Breeding and colony management | NC3Rs.

A retrospective assessment of reduction will be due by 28 February 2030

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use only mice. Rodents are the species with the lowest sensitivity of the nervous system likely to produce data predictive of the effect in humans. Mice are an established and reliable model for human immune responses. Consequently, there are a range of methods, reagents and testing kits for mouse immune responses that make them more suitable than other rodent models. In addition, there are well-established and reliable genetically modified models that enable us to study vaccine responses and side effects in detail and depth in mice that are not present in other rodents. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and how drugs work in the body based on mice, which provides the background to our studies. The use of these well-documented models means that adverse effects can be treated early and more appropriately mitigated.

We select the tumour models most similar (biologically, genetically and in drug response) to human cancer. Where the science permits we will use tumours under the skin, as those mice will have fewer health issues than other cancer models (e.g., directly injected into the veins), reducing the suffering of individual mice used for therapeutic studies. Some of the mice that are tumour recipients will be genetically altered to express certain markers for the detection of specific cell types. Others may be genetically altered to modify a gene suspected of being involved in the response (or lack of response) of the tumour to certain therapies.

Most mice in studies will be killed before they show any signs of illness, because the scientific endpoint of the study is reached before the cancer is too advanced, but mice will be killed if they do develop signs of cancer-related illness or drug-related harmful effects, to reduce pain, suffering, distress and lasting harm.

Infected mice may experience clinical signs of infection, such as weight loss, reduced activity and motility, difficulty to breathe, and in accessing water and food. Vaccinated mice will receive multiple treatments which carry a risk of adverse effects. Any animal exceeding or likely to exceed moderate severity or weight loss superior to 15% of the initial weight will be immediately killed by a Schedule 1 method.

Why can’t you use animals that are less sentient?

Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of infection, vaccination and cancer have been developed in mice, and there is a longstanding literature on immune responses, cancer biology and pharmacology based on mice, which provides the background to our studies. These models require adult animals, during this stage of life, the immune system is totally developed and can be challenged with different vaccine platforms. Aged mice may be used to mimic vaccine responses in late stages of life, e.g., old people.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have experience using genetically modified and unmodified mice utilising the immunisation protocols we intend to use under this license. Adverse effects with model proteins – that stimulate the immune system without the need for a pathogen - are rare and are similar to those induced by vaccination in humans. Some inflammation and discomfort may be induced with the use of adjuvants, a vaccine component which helps make the vaccine stronger and more effective, although this should be minimal. The use of genetically modified mice with defects in their immune system may produce an immune deficiency. However, as the mice are kept and bred in the animal unit under specific pathogen-free conditions and do not experience infection, the mice should be unaffected.

When pathogens are used these will be attenuated and the mice will be monitored appropriately. Attenuated pathogens mean that they are altered in a way that makes them less harmful or less able to cause disease, but still able to stimulate the immune system. The body temperature may be taken post-infection as a drop or rise in temperature could be a precursor to clinical signs. If that is the case, this will trigger the provision of wet mash and/or additional warmth via a heat pad or chamber before the signs are seen in the animal. Should the pathogen be used in a mouse strain which we have not

used before a pilot study will be carried out under guidance from our animal facilities to establish the most refined protocol.

Our laboratory and animal facilities have experience in producing bone marrow-transplanted mice and strict written protocols will be followed, which may include the administration of antibiotics.

We have been using tumour models and most of the procedures for years already and so the methods are already refined. However, we will scrutinise new guidance as it becomes available and will look to adopt new best practices when advances are published. For procedures we have not used before, we will use pilot studies to develop the protocol and evaluate the best method. As our understanding increases of the timescale for the spreading of tumours (metastasis) in our models, we will refine our endpoints for those studies to avoid mice suffering from the presence of metastases.

For mice receiving modified diet, those specifically susceptible to diabetes will be monitored to detect metabolic alterations or clinical signs of diabetes including excessive urine production and dehydration (rapid weight loss). Urine glucose will be measured if appropriate.

For prolonged oral treatment (daily more than 10 times), we plan to refine this method by guiding the mouse to consume the solutions by the oral route voluntarily rather than administration directly to the stomach.

We will use the guidance documents the NVS and the local AWERB 3Rs Committee have generated, such as the tamoxifen guidance document. Tamoxifen is a medicine that is often used to treat breast cancer in humans. However, in mouse studies, tamoxifen is used differently, mice are treated with tamoxifen to change specific genes in their bodies. This helps us understand how different genes affect the body and how diseases might develop or be treated.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We minimise suffering by adhering to best practice guidance, currently the “NCRI Guidelines for the welfare and use of animals in cancer research” by P.Workman, et al., Br. J. Cancer (2010) 102, 1555-1577.

3Rs resource library. https://nc3rs.org.uk/

ARRIVE guidelines: https://arriveguidelines.org/

NORECOPA's PREPARE guidelines: https://norecopa.no/prepare

For surgical procedures:

LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.).
http://www.lasa.co.uk/publications/

For recording and reporting on experiments:

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLOS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412

Recently published literature in indexed journals of animal experimentation.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Via Mailing lists for initiatives from the NC3Rs, information circulated locally by the Named Information Officer and Named Animal Care and Welfare Officers, and information from local annual 3Rs symposia. In addition, one member of our lab sits on the local AWERB 3Rs Committee, so we will have the latest advances in the 3Rs.

Changes to practice would be considered and pilot studies performed to ensure any changes do not compromise the ongoing science.

A retrospective assessment of refinement will be due by 28 February 2030

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?